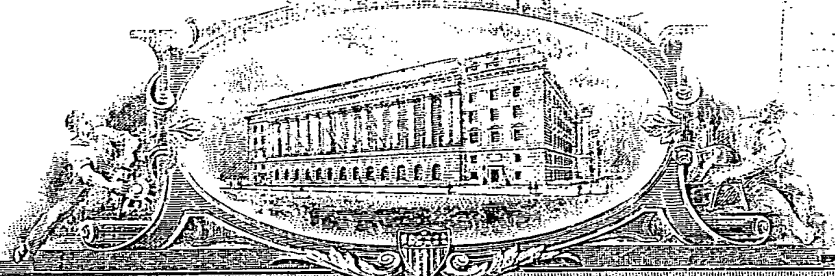


AP



# THE UNITED STATES OF AMERICA

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35 USC 111.**

**APPLICATION NUMBER: 60/072,089**

**FILING DATE: January 21, 1998**

**PCT APPLICATION NUMBER: PCT/US99/01170**

## **PRIORITY DOCUMENT**

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Customer Number: 000959

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of Kevin Croker and Seth Taylor

Attorney

Docket No. PKP-006-2

For:

Assistant Commissioner For Patents  
Box Provisional Patent Application  
Washington, DC 20231

CERTIFICATION UNDER 37 CFR 1.10

Date of Deposit: January 21, 1998

Mailing Label Number: EM284 255 263US

I hereby certify that this Cover Sheet for Filing Provisional Application (37 C.F.R. §1.51(2)(i)) and the documents referred to as attached therein are being deposited with the United States Postal Service on the date indicated above in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR 1.10 and addressed to the Assistant Commissioner for Patents, Box Provisional Patent Application, Washington, D.C. 20231.

Mark D. Russett

Name of Person Mailing Paper

Signature of Person Mailing Paper

COVER SHEET FOR FILING PROVISIONAL PATENT APPLICATION

Dear Sir:

The accompanying application, entitled \_\_\_\_\_,  
is a provisional patent application under 37 C.F.R. §1.51(a)(2) and §1.53(b)(2).

1. ☒ The name(s) and address(es) of the inventor(s) of this application is/are as follows:

	Last Name	First Name	Middle Initial	Residence
1	Croker	Kevin		
2	Taylor	Seth		Cambridge, MA
3				
4				

2. ☐ This invention was made by an agency of the United States Government or under contract with an agency of the United States Government. The name of the U.S. Government agency and the Government contract number are:

Agency: \_\_\_\_\_

Contract No.: \_\_\_\_\_

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3. ☒ The following documents are enclosed:

- ☒ 9 page(s) of specification
- ☒ 4 sheet(s) of drawings (Figs. 1-4)
- ☒ 1 page(s) of claims
- ☒ 1 page(s) of abstract

4. ☐ A verified statement to establish small entity status under 37 CFR 1.9 and 1.27 is enclosed.

5. ☐ An Assignment of the invention to \_\_\_\_\_ is enclosed.  
A check in the amount of \$40.00 for recording this assignment and a recordation form cover sheet (Form PTO 1595) are also enclosed.

6. ☒ The fee for filing this provisional application, as set forth in 37 CFR 1.16(k), is \$150.00.

- a. ☒ A check for this filing fee is enclosed.
- b. ☐ Charge the filing fee to Deposit Account No. 12-0080. (A duplicate copy of this sheet is enclosed.)
- c. ☐ The filing fee is not being paid at this time.


7. ☒ Please charge any fee deficiencies associated with this filing to Deposit Account No. 12-0080. A duplicate copy of this sheet is enclosed.

8. ☒ Please address all future communications to: **Customer Number: 000959** whose address is:

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Respectfully submitted,



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## GEL PAD ARRAYS AND METHODS AND SYSTEMS FOR MAKING THEM

### Related Applications

This application is related to commonly-owned U.S. Provisional Patent Application entitled "Gel Pad Arrays and Methods of Making and Using Them", filed on January 20, 1998. This application is also related to commonly-owned U.S. Provisional Patent Application entitled "Gel Pad Arrays on Flexible Tapes", filed on even date herewith. The contents of each of these provisional patent applications is hereby incorporated by reference.

### Background of the Invention

Rapid advances in the ability to accurately determine polynucleotide sequences, such as DNAs and RNAs from the genomes of organisms, has made possible the sequencing of huge quantities of polynucleotides. In recent years, the entire genomes of microorganisms, such as *Helicobacter pylori*, have been sequenced.

Traditional sequencing methods have relied on automated sequencing equipment which processes a polynucleotide strand one base at a time. A more recent approach, sequencing by hybridization (SBH), which could potentially increase sequencing throughput, relies on fragmenting a target polynucleotide into short segments; these short segments can be captured, for example on an ordered microarray of immobilized complementary single-stranded DNA probes, and the sequences of the target polynucleotide determined by analyzing the overlap of the sequences of the DNA probes bound to fragments of the target polynucleotide. See, for example, U.S. Patent No. 5,525,464 to Drmanac *et al.* Microarrays of DNA attached to a solid support have been prepared, see, for example, U.S. Patent 5,445,934 to Fodor *et al.*

Often, however, DNA microarrays are limited to analyzing nucleic acids in a single, fluid environment. An alternative to conventional DNA microarrays on a solid support is a microarray comprising biological molecules, such as DNA, attached to a matrix of crosslinked polymers known as gel pads. See, e.g., U.S. Patent No. 5,552,270 to Khrapko *et al.* Gel pads provide the ability to customize the micro-environment surrounding the DNA in each individual gel pad, which makes possible more sophisticated experiments in micro-array format.

However, although gel pads have certain advantages over convention micro-arrays, new types of arrays, and methods for making them, are needed.

### Summary of the Invention

This invention features gel pad arrays, e.g., arrays on a support, and methods for making and using them. The arrays can be used for sequencing by hybridization (e.g., where the pads include nucleic acid strands immobilized within the gel matrix), for cell based assays

(e.g., where the pads include, or are adjacent to and contacting, living cells), and for other uses which will be apparent to one of ordinary skill in the art.

In one aspect, the invention provides a method for preparing an array of gel pads. The method includes the steps of providing a first gel layer on a substrate; selectively removing portions of the first gel layer to create voids in the first gel layer; providing a second gel in the voids; and removing the first gel layer, such that an array of gel pads is provided.

In another embodiment, the invention provides a gel pad comprising a living cell; or an array of such gel pads. In another aspect, the invention provides a gel pad comprising a first gel layer and a second gel layer adjacent to and in contact with said first gel layer.

### Brief Description of the Drawings

Figure 1 depicts gel pads of the invention which include living cells.

Figure 2 depicts apparatus for preparing gel pads and transferring the pads to a substrate to form an array of gel pads.

Figure 3 depicts a system for manufacturing and testing a tape substrate with gel pads disposed thereon.

Figure 4 depicts an imager for use in quality assurance of a tape substrate having gel pads deposited thereon.

### Detailed Description of the Invention

This invention provides gel pads and gel pad arrays having a variety of uses, some of which are known in the art. The invention also provides methods for making the gel pads and gel pad arrays of the invention.

The term "gel pad" is known in the art and as used herein refers to a discrete portion of a gel disposed on a substrate such as a solid support, e.g., a plastic, glass, or metal substrate. The substrate can be any support suitable for supporting a gel pad, and can be rigid (e.g., a glass or plastic plate or sheet) or flexible (e.g., a tape), transparent (e.g., for performing optical measurements through the pad and substrate) or opaque. The properties of the support can be readily selected for use in any particular application. In preferred embodiments, the solid support is substantially non-reactive under conditions used to perform an assay or test procedure with the gel pad or gel pad array. An "array" can be any pattern of spaced-apart gel pads disposed on a substrate; arrays can be conveniently provided in a grid pattern, but other patterns can also be used. In preferred embodiments, a gel pad array according to the invention includes at least about 10 gel pads, more preferably at least about 50, 100, 500, 1000, 5000, or 10000 gel pads. In certain embodiments, the array is an array of gel pads of substantially equal size, thickness, density, and the like, e.g., to ensure that each

gel pad behaves consistently when contacted with a test mixture. In certain embodiments, however, the pads of a gel pad array can differ from one another; e.g., a mixed gel pad array can be constructed which includes more than one size or type of gel pad, e.g., gel pads made of different gel materials, or which entrap different species such as reagents or polynucleotide probes. In certain preferred embodiments, gel pads in an array are less than about 1 mm in diameter (or along a side, e.g., in the case of square gel pads), more preferably less than about 500 microns, still more preferably less than about 100, 75, 50, 25, 10, 5, or 1 micron in diameter.

A gel pad can have any convenient dimension for use in a particular assay. In preferred embodiments, a gel pad is thin enough, and porous enough, to permit rapid diffusion of at least certain reaction components into the gel pad when a solution or suspension is placed in contact with the gel pad. For example, in one embodiment, a gel pad array for use in sequencing by hybridization permits polynucleotide fragments from a sample mixture to diffuse (within a conveniently short time period) into the gel pads and hybridize to oligonucleotide capture sequences disposed within the gel pads. In certain preferred embodiments, a gel pad (e.g., in an array of gel pads) has a thickness of at least about 1, 5, 10, 20, 30, 40, 50 or 100 microns. In certain preferred embodiments, a gel pad (e.g., in an array of gel pads) has a thickness of less than about 1 millimeter, 500 microns, 200, 100, 50, 40, 30, 20, 10, 5, or 1 microns.

It will be appreciated from the foregoing that a gel pad can entrap additional chemical species, if desired, e.g., to perform assays with or within the gel pad. For example, gels which include DNA probes have been used for SBH (for example, U.S. Patent No. 5,552,270 to Khrapko *et al.*). Thus, a gel pad can be prepared such that a chemical species is trapped within the gel pad, or a desired species can be added after the gel pad has been prepared, e.g., by contacting a preformed gel pad with a solution of the reagent and allowing the reagent to diffuse into the gel pad. Examples of reagents which can be entrapped, suspended or dissolved in a gel pad include proteins, such as enzymes (e.g., ligases, which can be useful for positional SBH (see, e.g., Cantor, U.S. Patent Nos. 5,503,980 and 5,631,134)), polynucleotides, growth factors (e.g., for use with cells, e.g., see *infra*), salts and the like.

In one aspect, the invention provides methods for making gel pads and gel pad arrays. In certain preferred embodiments, gel pads and gel pad arrays can be conveniently prepared by use of "intelligent gels." Intelligent gels are gels which have properties which change (preferably reversibly) in response to changes in external conditions (for descriptions of certain intelligent gels, see, e.g., Kajiwarra *et al.*, "Synthetic Gels on the Move", *Nature*, vol. 355, pp. 208-209 (1992); Kwon *et al.*, "Electrically Erodible Polymer Gel for Controlled Release of Drugs", *Nature*, vol. 354, pp. 291-293 (1991); Suzuki *et al.*, "Phase Transition in Polymer Gels Induced by Visible Light", *Nature*, vol. 346, pp. 345-347 (1990); Osada *et al.*, "Intelligent Gels", *Scientific American*, pp. 82-87 (1993); R. Dagani, "Intelligent Gels," *Chem. Eng. News.*, June 9, 1997). Examples of intelligent gels include gels which become

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softer or firmer (e.g., solidify or liquefy) in response to changes in temperature, salt concentration (e.g., ionic strength), pH, exposure to radiation (e.g., ultraviolet (UV) radiation), presence or absence of a selected metal ion, electrical current, magnetic field, and the like. For example, a copolymer of poly(acrylic acid) and poly(N-isopropylacrylamide) has been reported to be temperature-sensitive, swelling at lower temperatures and collapsing at higher temperatures (Tanaka et al., *Faraday Discuss.* 101:201 (1995)). One of ordinary skill in the art will be able to select an intelligent gel with the desired properties for a selected application using no more than routine experimentation. In certain preferred embodiments, an intelligent gel for use in the present invention is responsive (e.g., liquefies) in response to an increase in temperature or irradiation with ultraviolet light.

In an illustrative embodiment, an intelligent gel can be used to prepare a gel pad array. The gel pads can comprise an intelligent gel, or the intelligent gel can be used as a form or mold to prepare a gel pad array. For example, in one embodiment, an intelligent gel which liquefies in response to UV irradiation is cast in a thin film on a substrate such as a glass plate. The gel can incorporate reagents, such as polynucleotide probes for capturing fragments of DNA from a solution; alternatively, such reagents can be added after the array has been formed. The gel is allowed to cool and solidify. The gel layer is then masked, e.g., with a mask such as is conventionally used in photolithography; the mask protects gel portions in an array configuration on the substrate (e.g., a 100 x 100 array of gel pads). The masked gel layer is exposed to ultraviolet light. The exposed portions of the gel liquefy and are poured off or washed off with a suitable solvent, without disturbing the array. After irradiation and removal of the mask, an array of gel pads is obtained.

Alternatively, an intelligent gel can be used as a mold or form for preparing a gel pad array. An intelligent gel which is temperature-responsive is cast on a substrate. The gel layer is then exposed to a laser, which is rasters over the gel layer and irradiates selected gel portions in the configuration of an array (see also Patent Cooperation Treaty Publication WO95/04834). The portions of the gel pad which are exposed to the laser source are heated and become liquefied; the liquefied portions are removed, e.g., by gentle washing. (The gel layer could be selectively heated by other means, such as an array of heated wires or probes which are brought near to, or into contact with, the surface of the gel layer.) The gel layer now has an array of "holes" formed by removal of the gel portions exposed to the laser source. These "holes" can be filled with a second gel (which can be a different intelligent gel or a conventional gel, such as polyacrylamide); the second gel is permitted to solidify, forming an array of gel pads within the intelligent gel layer. The slide is then heated (e.g., by placing the substrate in a warming bath or a warming oven) to liquefy the intelligent gel layer, which is then removed by washing or pouring off the liquefied material. An array of gel pads remains on the substrate and can be further processed, if desired.

It will be appreciated that the methods of using intelligent gels to prepare gel pad arrays will have many applications. The mild conditions employed can be tailored to the

preparation of a wide variety of intelligent and conventional gel pad arrays, preferably without degradation of sensitive reagents, such as polynucleotide probes, which may be present in the gel layer. Methods for preparing gel pads, e.g., such as conventionally known or described herein, can be combined, if desired.

Furthermore, the use of intelligent gels in gel pad arrays provides additional advantages. For example, an intelligent gel pad can be provided which swells in response to a change, such as the presence of an analyte of interest. For example, an intelligent gel which swells in response to pH changes is provided in a gel pad on a support. The gel pad includes glucose oxidase. The reaction of glucose oxidase with glucose produces gluconic acid, lowering the pH of the gel. Thus, in the presence of glucose in a sample solution which is brought into contact with the gel pad, the gel pad will shrink. A gel pad can be provided adjacent to a piezocrystal, such that changes in gel pad swelling produce a piezoelectric signal, which can be detected and correlated with the glucose concentration.

Gel pad arrays can also be prepared by treating the surface of the substrate to create a pattern of alternating hydrophobic and hydrophilic sites on the surface. For example, a glass surface can be silated with a conventional silating reagent to prepare a patterned surface having hydrophobic and hydrophilic portions. A gel, such as an intelligent gel, is then poured onto the surface. A hydrophobic gel will be repelled by a hydrophilic surface, while a hydrophilic gel will be repelled by a hydrophobic surface. A patterned surface can be used to urge the liquefied gel into a pre-selected pattern on the substrate, thereby forming a gel pad array.

In another embodiment, a gel pad (e.g., in an array) can be prepared through the use of a derivatized monomer unit, followed by formation of the gel pad by polymerization of the monomer. For example, acrylic acid can be readily derivatized with a polynucleotide (e.g., a probe for use in SBH); for example, a polynucleotide can be coupled to acrylic acid through the use of a conventional coupling reagent such as dicyclohexylcarbodiimide (DCC) (or a water-soluble derivative thereof such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, EDC). A spacer or linker moiety can be used to increase the distance between the acrylate monomer and the polynucleotide, if desired, e.g., to increase mobility of the polynucleotide in the polymer). The resulting acrylic ester of the polynucleotide can then be disposed in an array format on a substrate, e.g., by dispensing a solution of the acrylic ester through a nozzle or array of nozzles (such as conventional piezoelectric ink-jet printing nozzles; see also Patent Cooperation treaty Publication WO95/04594). Alternatively, an array format can be provided by using a cast or mold. The array of droplets is then polymerized *in situ* to provide an array of gel pads which incorporate a polynucleotide covalently bound to the gel polymer.

Another method for preparing a gel pad array comprises preparing individual gel pads, or sub-arrays of gel pads, on a first substrate, and then transferring the individual gel pads to a second substrate, in array format, to prepare a gel pad array on the second substrate. This method can be used to substantially avoid covalent attachment of the gel pad to the



second substrate. In addition, the gel pads prepared on the first substrate can be examined to ensure quality of the individual gel pads, and faulty gel pads (e.g., of the wrong shape or size) can be removed before the final array is prepared on the second substrate. This procedure can prevent the formation of arrays which contain faulty or non-standard gel pads. Moreover, the gel pads can be further processed (e.g., washed, imparted with an additional component such as a protein, and the like) prior to transfer of the gel pads from the first substrate to the array format on the second substrate.

For example, gel pads can be prepared on a first substrate, such as a tape, and then transferred to a second substrate, such as a glass or plastic plate, in an array format, to provide a gel pad array on the second substrate. The gel pads can be transferred by contacting the first and second substrates, e.g., by pressing the first substrate against the second substrate, such that the gel pads are transferred from the first substrate to the second substrate. The transfer can be facilitated by making using first and second substrates which have different surfaces, e.g., a hydrophobic first substrate and a hydrophilic second substrate; in this example, a hydrophilic gel pad will be more adherent to the second substrate and will be transferred from the first substrate to the second substrate when the two substrates are pressed together.

The transfer can be facilitated in other ways. For example, the gel pad can be electrically charged, and the electric charge of the first and/or second substrate can be adjusted such that the gel pad is repelled from the first substrate and attracted to the second substrate. In another embodiment, an intelligent gel can be employed to facilitate the transfer. For example, the first substrate can be coated with a thin layer of an intelligent gel such as described above, prior to the deposition of the gel pads on the first substrate. When the first and second substrates are placed into close contact, the intelligent gel can be liquefied. For example, for an intelligent gel, such as "Smart Hydrogel", which liquefies at cooler temperatures, liquefaction can be accomplished by cooling the first and/or second substrate. When the intelligent gel is liquefied, the gel pads disposed on the intelligent gel layer on the first substrate cannot adhere to the first substrate, and are transferred to the second substrate. Similarly, for other intelligent gels, the first and/or second substrates (or selected portions thereof) can be heated, subjected to an electric current, contacted with a solution having a high pH or salt concentration, and the like, to liquefy or soften the intelligent and thereby release the gel pads from the first substrate and adhere the pads to the second substrate.

In one exemplary embodiment of this method of the invention, illustrated in Figure 2, a system for creating gel pads on a first substrate and transferring the pads to an array format on a second substrate includes tape winding reels 10, 12 between which a tape 15 (e.g., a polystyrene tape) (first substrate) is passed. The tape can optionally be used for information storage, e.g., by coating with a conventional magnetic oxide layer. As the tape passes from the first tape reel 10, it is guided along a tape path by guide wheel 20, past a gel pad dispenser

(e.g., a nozzle, not shown, which deposits a solution of the gel monomer, which can be polymerized *in situ*) which deposits gel pads along the tape 15. The gel pads can optionally be washed, e.g., by spraying the pads with a buffer solution followed by air-drying, if desired. As the tape moves beyond the gel pad dispenser, each pad can be assayed to ensure the quality of the pads deposited (see *infra* and Figures 3 and 4). Defective pads are noted and the location stored and tracked, e.g., in a computer memory. The tape passes over a temperature-controlled head 30, which can be heated (or cooled) according to the properties of the gel pad employed. The tape head is controlled by a microcomputer which first ensures that the gel pad currently under the head is not defective; defective pads are passed unchanged over the tape head and toward tape takeup reel 12, where they are collected. If the gel pad under the tape head is of acceptable quality, the microcomputer positions the tape head over the second substrate 40 (or moves the second substrate 40 under the tape head) to the correct location for the next pad in the array on second substrate 40. The tape head is then urged against second substrate 40 and the temperature is changed (e.g., the tip of the tape head is heated). In response to the change in temperature, the gel pad is dissociated from the tape 15 as the tape head is pressed against the surface of a second substrate 40, and the gel pad is transferred to the substrate 40. Used tape is collected past guide wheel 22 to takeup reel 12, and can be cleaned (e.g., to remove defective pads and any residue) and reused for further array preparations.

Gel pads can be transferred from the first substrate in groups, e.g., in a row or rows, rather than one at a time, as illustrated above. Furthermore, the second substrate can also be a tape, rather than a rigid substrate; in this case, the second substrate tape could be urged against the first (tape) substrate by means of a roller or other tape transport mechanism. It will also be appreciated by the ordinarily skilled artisan, in light of the disclosure herein, that systems such as described herein can be used to effect the transfer of arrays of nucleotides (including, but not limited to DNA, RNA, and peptide nucleic acids) from one substrate to another, with or with concomitant transfer of a gel pad. Thus, for example, DNA can be transferred from one substrate to another, e.g., electrostatically, as described above.

A manufacturing system for preparing a first (tape) substrate with gel pads deposited thereon is shown in Figure 3. The system includes tape reels which serve to pass tape, at the urging of a precision stepper, under a dispenser, which provides pads on the first substrate. The pads are polymerized at a polymerization station, followed by washing at a wash station to remove impurities, unpolymerized monomers, and the like. The QA station can include a spectrophotometric instrument for determining the size, shape, and quality of the pads deposited on the substrate. In one embodiment, a charge-coupled device camera can be used to detect fluorescence, e.g., in response to a laser source, of a fluorescent molecule which is incorporated in the gel layer (and can optionally be removed at a later processing step, e.g., a second washing station). An exemplary imaging system is shown in more detail in Figure 4. If a magnetic layer is included in the tape substrate, the tape can be encoded with information

such as date of manufacture, location of defective pads, and the like. The tape is taken up on a take-up reel and stored for later transfer of the pads to an array on a second substrate, e.g., as described above. To prevent destruction of the pads as the tape is wound on the take-up reel, the tape can be ridged, for example as shown in the upper inset of Figure 2, to prevent crushing of the pads.

In another embodiment, the first substrate can be a roller, e.g., a cylindrical element. Gel pads can be provided on the surface of the roller as the roller is rotated by a motor; the gel pads on the roller can be washed and assayed as described above. The gel pads are then transferred from the roller surface to the second substrate (which could be rigid or a flexible tape) as described above. After the gel pads are transferred to the second substrate, the roller surface as it rotates preferably passes through a cleaning apparatus. The roller surface can then be cleaned with each revolution to prevent contamination of gel pads with residue from preceding preparations. This system advantageously can provide continuous, rather than batch, operation.

The invention also provides multi-layered gel pad constructs. For example, in one aspect, the invention provides a gel pad which comprises at least two gel layers in contact with each other, e.g., a first gel layer on which is disposed a second gel layer, or first gel layer adjacent to and in contact with a second gel layer. A multi-layer gel pad of the invention can have two, three, four, or more layers, although greater numbers of layers will generally require more effort to prepare. The multi-layer gel pads of the invention can be configured to provide a variety of functions. For example, a first gel pad layer can include a polynucleotide (e.g., a probe for performing SBH) within the first gel matrix. A second gel layer can be disposed over and covering the first gel layer; the second gel layer can be a gel having an effective pore size small enough to prevent the diffusion of high-molecular-weight substances, such as proteins. The second layer thus serves as an effective barrier to prevent diffusion of substances, e.g., proteins, from a sample solution into the first gel layer, or from the first gel layer into solution. The multi-layer gel pad can prevent interference from sample constituents, or can prevent the loss of valuable components from the first gel layer.

In another embodiment, a first gel layer can be formed with low ionic strength, e.g., an ionic strength lower than the ionic strength of a sample solution to be applied to the gel pad array. A second, protective or filtering gel layer covers and encapsulates the first gel pad layer. The low ionic strength of the first gel layer promotes osmotic movement of sample components into the first gel layer, thereby increasing the sensitivity of the first gel layer for a sample component of interest.

A multi-layer gel pad can be constructed by methods known in the art for the preparation of single-layer gel pads, or by the methods described herein. It will be appreciated that in certain embodiments, it is preferred to maintain registration between the layers of the multi-layer gel pad, e.g., in certain embodiments, it is preferred to place a second gel layer directly atop a first gel layer. The use of a mold or form can be useful in this

embodiment, because molds can provide good registration between layers.. A particularly useful method for preparing a multi-layer gel pad array is the intelligent gel "molding" or "forming" layer methodology described above.

In still another embodiment, the invention provides gel pads which include living cells (referred to herein as "cell pads"). In one embodiment, the gel pad of the invention is a multi-layered gel pad, having a first layer without cells, and second layer which includes cells (e.g., bacterial or eukaryotic cells). (Alternatively, cells can be grown on top of a gel layer, without being immobilized within a second gel layer). Exemplary embodiments are shown in Figure 1. Figure 1A depicts a first gel layer disposed adjacent a second, cell-containing gel layer. Figure 1B depicts cells immobilized in a second gel layer which encapsulates a first gel layer. Figure 1C shows cells maintained on the surface of a gel layer. The cells can be maintained in culture. This embodiment, provides a useful assay format for performing cell based assays in an array format. For example, the first gel layer could include detection means for detecting the presence (or absence) of a cell constituent (such as DNA) or a product of cellular metabolism (such as proteins, or products of transcription). For example, the cells in one layer can secrete molecules, such as growth factors, which can be monitored by the use of capture molecules in another layer of the multi-layer gel pad. The cells can also be lysed and cellular components measured. Thus, the response of the cells to a stimulus, such as addition of a growth factor, a toxin, a drug, or the like, can be monitored in a convenient and easily handled format.

Cell pads can also be configured to permit cells in one pad to secrete molecules which influence the growth of other cells in adjacent pads, e.g., an autocrine system. Thus, complex cell-based assays can be reduces to microscale format.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

The contents of all publications and patent applications described herein are hereby incorporated by reference.

Other embodiments are within the following claims.

What is claimed is:

1. A method for preparing an array of gel pads, the method comprising:  
providing a first gel layer on a substrate;  
selectively removing portions of the first gel layer to create voids in the first gel layer;  
5 providing a second gel in the voids; and  
removing the first gel layer, such that an array of gel pads is provided.

2. The method of claim 1, wherein the first gel layer comprises an intelligent gel.

10 3. A gel pad comprising a living cell.

4. An array of the gel pads of claim 3.

5. A gel pad comprising a first gel layer and a second gel layer adjacent to and in contact  
15 with said first gel layer.

6. The gel pad of claim 5, wherein at least one of the first gel layer and the second gel  
layer comprises an intelligent gel.

20 7. A method for preparing a gel pad array, the method comprising:  
preparing gel pads on a first substrate; and  
transferring the gel pads from the first substrate to a second substrate in an array  
format, thereby preparing a gel pad array.

25 8. The method of claim 7, wherein the first substrate is coated with an intelligent gel.

**Abstract**

Gel pads and gel pad arrays, and methods for making and using them, are disclosed. The gel pads preferably comprise an intelligent gel.

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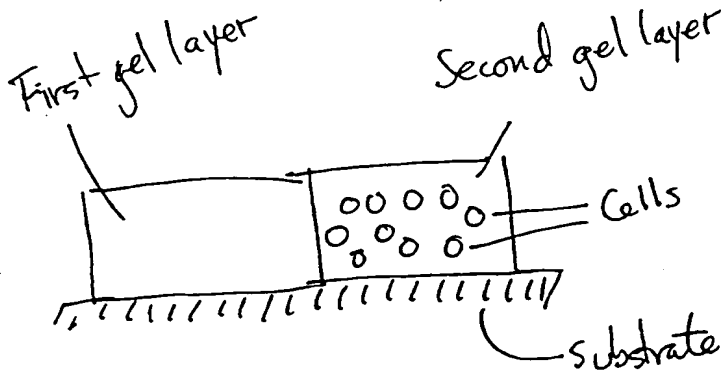


Figure 1A

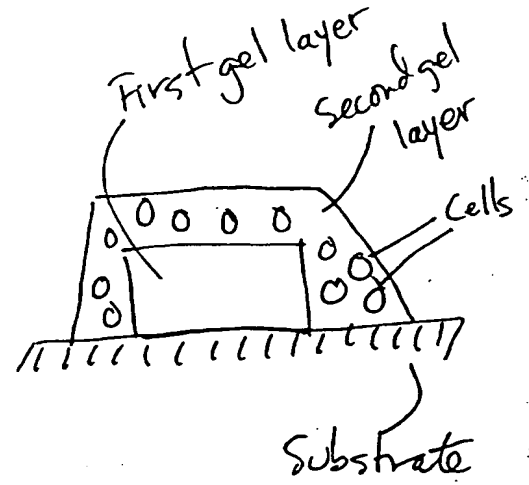


Figure 1B

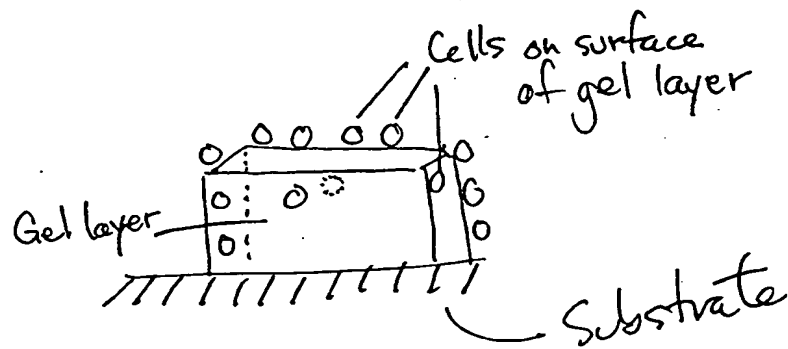


Figure 1C

USPTO 6802,009

Master Array Tape Driver  
Gel pad tape manufactured in a separate device.

Tape with Gel Pads Attached

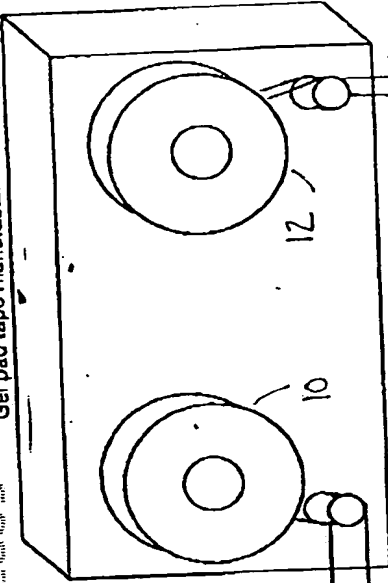
Top View

End View

Gel Pads

QC testing of gel pads

Could include:  
Size analysis, integrity, composition  
etc....



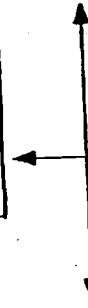
Guide Wheels

Bad pads remain

Z Axis servo to move head assembly

Heating/Cooling Element for pad release

Array Substrate with Gel pads attached



Substrate moves in X, Y or Z directions.

NOT TO SCALE

Figure 2



861210 68022009

# Manufacturing Enclosure

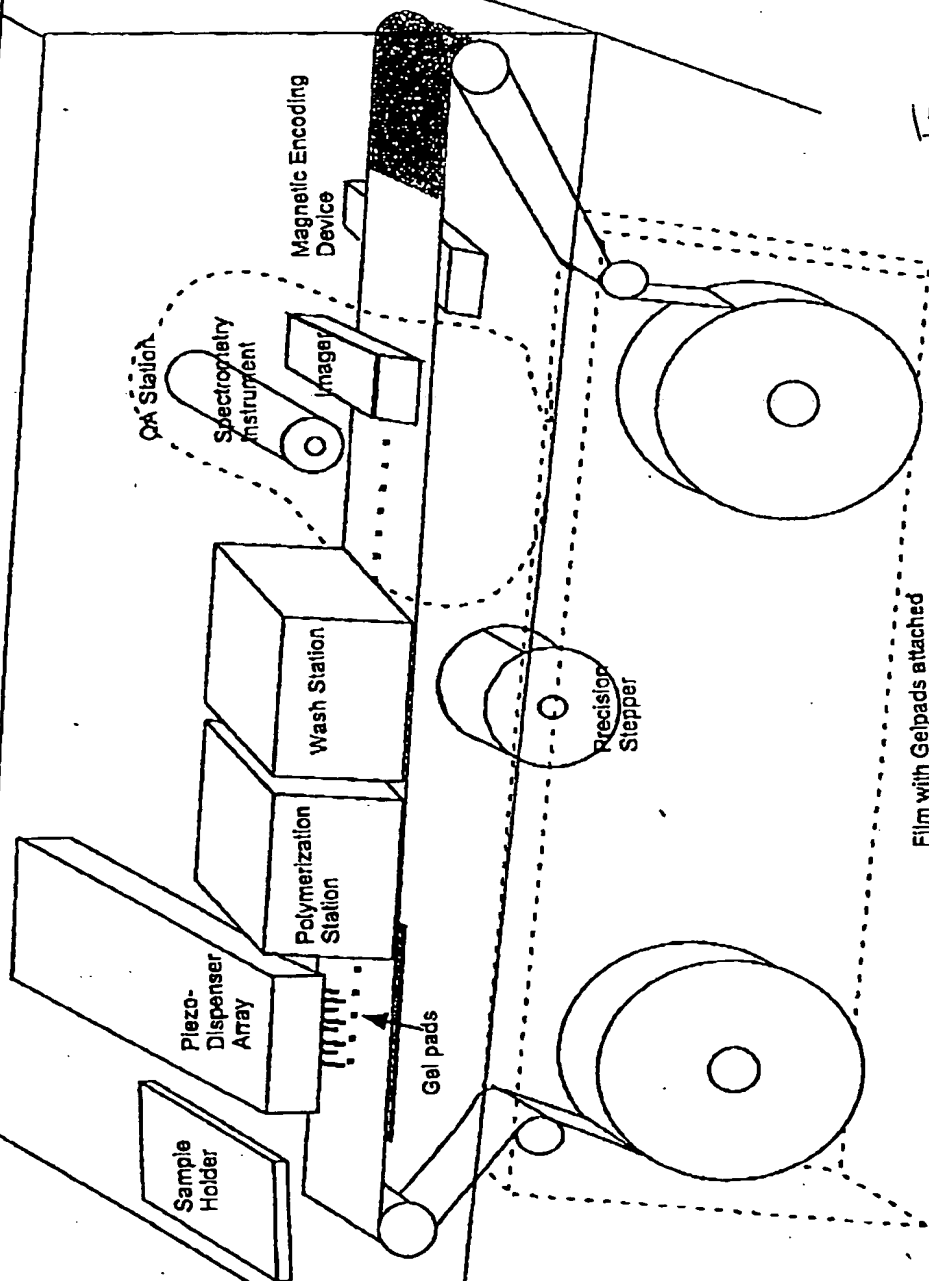
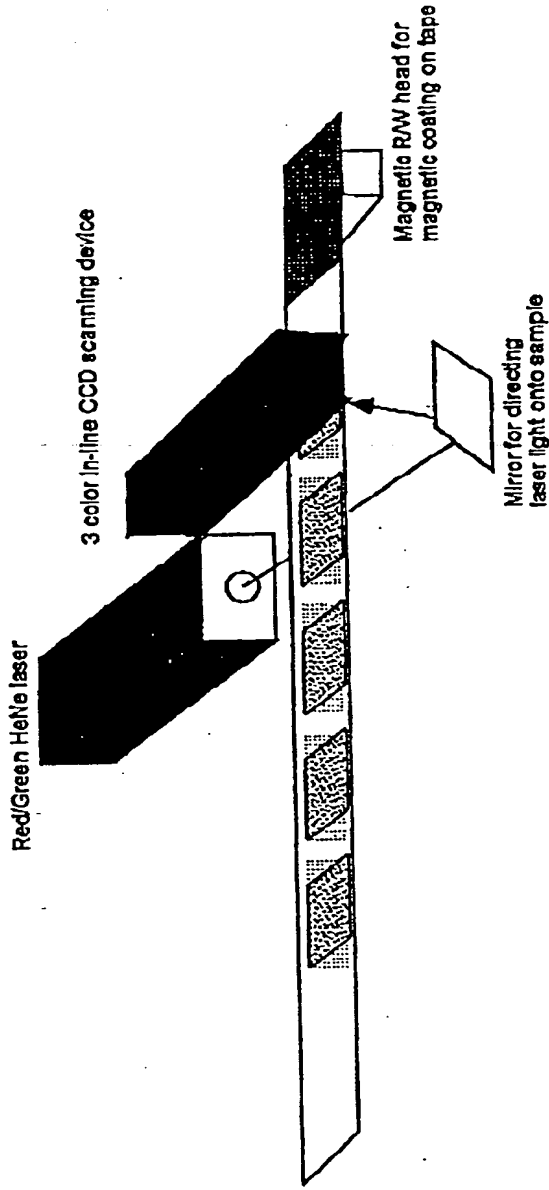


Figure 3

Instrument may contain additional fluids (sample) and liquid handling devices.

Gel Pad Processor - NOT TO SCALE

86T210-6802/009



**Overall Description:**  
 A high resolution 3 line color CCD  
 be mounted above the tape. The tape  
 then be incremented forward while the CCD scanned  
 the fluorescing gel arrays which have been excited by a laser  
 directed below the tape. A selectable filter wheel for the  
 laser could also be implemented.  
 A magnetic head be mounted for reading and writing data  
 to the tape.

Figure 4

Component: 4 - Inline Color CCD Imager	Not to Scale
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